PATENT
Customer No. 22,852
Attorney Docket No. 01288.0016

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
CAROLYN ELIZABETH LISTER et all.	)	Group Art Unit: 1638
	)	
Serial No.: 09/890,064	)	Examiner: Georgia L. Helmer, Ph.D.
	)	
Filed: 31 October 2001	)	
	)	
FOR: TRANSFORMATION AND REGENERATION OF ALLIUM PLANTS	)	**SMALL ENTITY**

Commissioner for Patents
Washington, DC 20231

Sir:

DECLARATION UNDER 37 C.F.R. § 1.132

I, Colin Charles Eady of Christchurch, New Zealand, do hereby make the following declaration:

1. I am a senior research scientist at Crop & Food Research where I have been employed for the last ten years as a plant geneticist within the germplasm enhancement team.

Qualifications

I have 14 years postdoctoral research experience in areas of plant biotechnology.

I graduated with honours in zoology and genetics from the University of Sheffield in the UK and then went on to gain a post graduate certificate in education and a doctor of philosophy in biotechnology. My early post-doctoral expertise involved research into plant gene regulation at the University of Lancashire, plant–microbe interactions at Nottingham University, and the safety of transgenes in the environment at Leicester University, where I was among the first to publish information on GM plant safety issues.

In 1993 I got the opportunity to research onion biotechnology at Crop & Food Research and in 1999 our team patented the method for transforming onion, or *Allium*, species.

Since then we have become world leaders in this field and are currently running projects, involving the genetic modification of onions, on:

- Enhancing our understanding of sulfur regulation in onions
- Enhancing our understanding of carbohydrate regulation in onions
- Evaluating antimicrobial genes in onions
- Extending transformation systems to other commercial allium species
- Developing herbicide resistant onion lines.

In addition to running or jointly running these projects, I also lecture on population biology and crop evolution/breeding at Lincoln University. I am also project leader under the Advanced Agri-biotechnology theme within the National Centre for Advanced Bio-protection Technologies.

2. I am familiar with the claims of U.S. Patent Application Serial No. 09/890,064, the Office Actions from the U.S. Patent and Trademark Office ("USPTO") pertaining to this Application, and EP0486234 by Bidney et al. ("Bidney"). I understand that the Examiner believes that the Bidney reference makes obvious the claims of this invention. It is my opinion, however, that Bidney neither describes nor makes obvious the claims of the present application.

3. Before our work, no one has successfully transformed Allium. To my knowledge, only a single example exists, other than Bidney's, that uses a Bidney-like method for transformation, and this example transformed strawberry. (Cordero de Mesa, M.; Jimenez-Bermudez, S.; Pliego-Alfaro, F.; Quesada, M. A.; Mercado, J. A. **Agrobacterium** cells as **microprojectile** coating: a novel approach to enhance stable transformation rates in strawberry. Australian Journal of Plant Physiology 27 (12): 1093-1100 2000; annexed as Exhibit CE1). All other published transformation attempts to use a protocol similar to that in Bidney failed. In fact, they suggest that such a transformation technique is detrimental to recovery of transgenic material (Teixeira da Silva, J. A.; Fukai, S. Four gene introduction methods affect the shoot regeneration and localization of transgene expression in greenhouse stem explants and in vitro-grown chrysanthemum stem thin cell layers. African Journal of Biotechnology 2 (5) : 114-123 2003); annexed as Exhibit CE2.

4. Even Bidney himself switched techniques just one year after development of the method described in the Bidney reference cited by the Examiner. This is significant because he switched even for his preferred plant, sunflower. (**Bidney, D. L.**; Scelonge, C. J.; Malone-Schoneberg, J. B. Transformed progeny can be recovered from chimeric plants regenerated from Agrobacterium tumefaciens treated embryonic axes of sunflower.

Proceedings of the 13th International Sunflower Conference Volume 2, Pisa, Italy, 7-11 September 1992. : 1408-1412 1992; annexed as Exhibit CE3). [DELETE - This trend has continued in his more recent references (Gordon-Kamm B.; Tao Y. M.; Dilkes B.; et al. Using genes that stimulate the cell cycle to improve maize transformation. Plant Biotechnology 2002 and Beyond. Proceedings of the 10th IAPTC&B Congress, Orlando, Florida, USA, June 23-28, 2002, annexed as Exhibit CE4.]

5. Thus, in the 12 years since the Bidney reference cited by the examiner, the method was rarely used generally, even more rarely used "successfully" (and only in a particularly amenable species), and never used for onion transformation.

6. Bidney states that the method described in that reference can be used for a whole gamut of species - roughly 75, using any one of 11 different tissue explants. It should be noted that this yields a total of 825 different permutations for possible regeneration. Bidney though provides only one transformation example, *i.e.*, sunflower. As such, the reference is so vague that it would be impossible for one skilled in the art to know which explant of choice should be used for which species.

7. Bidney states "The transformed plant cells produced by the practice of this invention are then suitable for regeneration by art-recognized techniques." At that time, however, and up until submission of the present application, there was in fact no art-recognized technique for the regeneration of transformed onion cells. Indeed the present application using regeneration of onion immature embryos without passage through a callus phase is the first such example of that technique in onions and as such is novel and inventive over the teaching of Bidney. This is especially relevant because from the time of the Bidney citation to the present application, the so-called 'normal' approach for transformation was to pre-culture explants with a passage through a callus phase. It was not 'the art' to use immature embryos without pre-culture and passage through a callus phase. In fact, a recently published book highlights this point. In "transgenic plants and crops," edited by Khachatourians, G.G. et al 2002, published by Marcel Dekker Inc., the transformation of 34 different crop species has been reviewed and only the chapter on onions, authored by me, directly uses immature embryos without pre-culture and passage through a callus phase. I believe this demonstrates that this Application discloses a totally different technique to what someone skilled in the art would attempt.

8. With respect to the article in Plant Cell Reports (1996) 15:958-962., I declare that the technique disclosed in that article failed to produce viable transgenic whole plants. I

believe that the reason for this was that the particle bombardment, though optimized for transient expression, may have been detrimental to cell survival and that the media used at the time was less than optimal for the recovery of transgenic cells.

9. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 27/01/04.

By: _____

Colin Eady

Witnessed by:

Signature: _____

Name: DASA VUKELIC

Address: Christchurch

Dasa Vukelic
Solicitor
CHRISTCHURCH

Occupation: _____